



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008138

OCT 26 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 053201, Inhalation Teratology Study in Rabbits
with Methyl Bromide

Tox. Chem. No.: 555
Project No.: 01807
Record No. : 268428

FROM: Melba S. Morrow, D.V.M. *M.S. Morrow*
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CONCLUSIONS:

The inhalation teratology study conducted in rabbits for methyl bromide has been classified as core minimum. The maternal NOEL is 40 ppm and the maternal LOEL is 80ppm based on the presence of decreased appetite, lethargy, right side head tilt, ataxia and lateral recumbency.

The developmental NOEL is also 40 ppm and the developmental LOEL is 80 ppm based on the presence of agenesis of the gall bladder, increased incidence of fused sternebrae and decreased fetal body weight.

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity (Inhalation)
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID No.: 415804-01
Caswell No.: 555

Test Material: Methyl Bromide

Synonyms: Bromomethane

Sponsor: Methyl Bromide Industry Panel

Study Number(s): K-00681-033

Testing Facility: Toxicology Research Laboratory Health and
Environmental Sciences
Dow Chemical
Midland, Michigan

Title of Report: Methyl Bromide Inhalation Teratology Study in
New Zealand White Rabbits

Author(s): Breslin, Zablony, Bradley and Lomax

Report Issued: June 18, 1990

Conclusions: The maternal NOEL = 40 ppm, the maternal LOEL = 80 ppm, based on decreased appetite, lethargy, right side head tilt, ataxia and lateral recumbency. The developmental NOEL was 40 ppm and the developmental LOEL was 80 ppm, based on the presence of agenesis of the gall bladder, increased incidence of fused sternbrae and decreased fetal body weights.

Core Classification: Minimum

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A. Materials

Test Compound: Purity: 99.6%
Description: Gas
Lot No.: 041889

Test Animal(s): Species: Rabbit
Strain: New Zealand White
Source: Hazleton - Duchland, Inc.
Age: Adult
Weight: approximately 3.5 - 4.5 kg

B. Study Design

This study was designed to assess the developmental toxicity potential of methyl bromide when administered by inhalation to rabbits on gestation days 7 through 19, inclusive.

Mating

Mating was conducted using artificial insemination. Three weeks prior to insemination, the rabbits were injected with 50 I.U. of HCG in order to synchronize estrous. On the day of insemination, the rabbits were reinjected with 100 I.U. of HCG to induce ovulation.

Group Arrangement:

Test Group	Dose Level (ppm)	Number Assigned
Control	0	26
Low Dose	20	26
Mid Dose	40	26
High Dose	80	26

Dosing:

The test compound was administered by inhalation at the above concentrations for a daily exposure time of 6 hours on gestation days 7-19.

The exposure chambers used in the study were a 1.6 meter cube with a pyramidal top. The test material was collected into bags of Saran film and the gas from these bags was metered into a J - tube at a constant rate. Compressed air was simultaneously directed into the main chamber airstream. Here the gas was diluted to the desired concentration. Air flow was determined with a flowmeter. The temperature and relative humidity were maintained at approximately 22° and 50%, respectively. Air, temperature and relative humidity were recorded at the end of each six hour exposure period.

Using a gas chromatograph with a nickle column packed with Tenax GC mesh and flame ionization detector, the analytical concentration of methyl bromide in each chamber was determined on an average of 14 times per exposure period. A nominal concentration could not be determined, since the test material was taken directly from the gas cylinder and placed in the bags of Saran film.

The distribution of methyl bromide within the breathing zone was determined from 9 sampling points along with the reference points within the breathing zone. This was done prior to exposing the animals and at one time during the study when the animals were inside the chamber.

Observations

The animals were checked for mortality or abnormal condition from day 0 to day 28 (or the day of delivery). Dams were sacrificed on day 28 of gestation, with the exception of one rabbit that delivered on gestation day 27. Examinations at sacrifice consisted of observation for obvious structural and pathologic changes; recording of maternal liver, lung, kidney, brain and gravid uterus weights; and collecting sections of maternal liver, kidney, lung, brain and nasal turbinates. The uterine horns were exteriorized and the number and position of fetuses in utero were recorded. Other observations included the number of live and dead fetuses, the number and position of resorption sites, the number of corpora lutea, the sex and body weight of each fetus and any external alterations.

For non-pregnant animals, each uterus was stained with a 10% solution of sodium sulfide to examine for evidence of early resorption.

The fetuses were euthanized with a subcutaneous injection of T-61 and were examined for external malformations. A low power stereomicroscope was used to examine the fetuses for visceral alterations. After evisceration, the fetuses were stained with alizarin red-S and were examined for skeletal alterations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis

Maternal body weights, body weight gain, fetal body weights, gravid uterine weights and absolute and relative organ weights were evaluated using Bartlett's test for equality of variances. A parametric or nonparametric analysis of variance (ANOVA) was performed based on the outcome of the Bartlett's test. If the ANOVA was significant, analysis by Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Censored Wilcoxon test with Bonferroni's correction were used to

analyze implantation loss, resorptions and fetal alterations among litters and among the fetal population. A nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction were used to evaluate the number of corpora lutea, the number of implants and the litter size. Fisher's exact probability test was used to analyze pregnancy rate and the sex ratio was analyzed using a binomial distribution test. Statistical outliers were identified but were not excluded from the data analyses.

Compliance

A signed Statement of Confidentiality Claim was not provided.

A signed Statement of compliance with EPA GLP's was provided. (Dated June 6, 1990)

A signed Quality Assurance Statement was provided. (Dated June 6, 1990)

C. Results

1. Maternal Toxicity:

Toxicity was observed at the 80 ppm exposure level. One doe in this treatment group delivered on gestation day 27.

Mortality: No deaths were reported during the study.

Clinical Observations

Clinical signs of toxicity observed in the high dose group included decreased fecal output, lethargy, right-side head tilt, ataxia, and lateral recumbency. No clinical signs of toxicity were present in the other two treatment groups.

Body Weight

There appeared to be a treatment related, but not dose related, decrease in body weight in the maternal animals in the high dose group (See Table I).

The values provided in the table are all mean values so expected additive values are not exact. In addition, the values for body weight that were reported in the summary do not correspond to the values in the raw data tables for the HDT group (See Table II).

Table I: Body Weight Gains (grams)^a

Group	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains	
					Dosing P. ¹	Entire ²
Control	95.8	101.0	46.0	273.7	-358.7	-186.2
LDT	141.3	84.3	-48.1	186.1	-364.8	-263.0
MDT	127.3	97.5	53.4	276.5	-312.2	-133.2
HDT	83.6	-10.5	16.4	113.4	-423.8	-299.9

¹ = corrected body weight gain for dosing period = body weight gain for dosing period minus gravid uterus weight.

² = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.

a = Data extracted from tables provided in the summary.

The following table depicts the reported weight discrepancies that existed between the raw data and the summary of the study.:

Table II: Body Weights for High Dose Group

Source	<u>Body weights(g)</u>						
	<u>Day</u>						
	0	7	10	13	16	20	28
Summary	3921	4005.5	3979.8	3968.6	3991.4	4010.6	4004.9
Raw data	3944	4019.9	3996.9	3988.9	4003.6	4034.0	4030.0

Food Consumption

No food consumption data were supplied by the sponsor. This omission would not be significant in that the rabbits are not receiving the test compound in the diet. Additionally, palatability would not be a factor in determining whether the animals received an adequate dose of the compound. The report did not indicate whether the food was left in the chamber after exposure nor whether decreased food consumption was present as a sign of toxicity.

Gross Pathological Observations

No gross pathological changes were observed by the investigators in the maternal animals.

Cesarean section Observations

A low pregnancy rate was observed in the LDT (20 ppm) but was unrelated to the test material and was reported as resulting from the artificial insemination technique that was employed. The number of resorptions were not designated as occurring early or late; the number in the following table represents the total number of resorptions.

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Table III: Cesarean Section observations^a

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	26	26	26	26
#Animals Mated/Inseminated	26	26	26	26
Pregnancy Rate (%)	80.8	57.7	73.1	76.9
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	5	11	7	6
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	1
Total Corpora Lutea				
Corpora Lutea/dam	11.8	12.9	10.8	10.9
Total Implantation				
Implantations/Dam	9.5	10.6	8.7	9.2
Total Live Fetuses				
Live Fetuses/Dam	9.0	9.1	7.5	8.4
Total Resorptions				
Resorptions/Dam	0.5	1.5	1.2	0.8
Total Dead Fetuses				
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	31.8	32.2	35.0	30.4
Preimplantation Loss(%)	18.2	17.7	21.2	16.1
Postimplantation Loss(%)	5.0	13.8	13.3	8.6
Sex Ratio (% Male)	48.9	55.5	53.8	47.8

^a = Data extracted from tables provided in the summary.

2. Developmental Toxicity

No fetal deaths were reported in any of the groups. Mean fetal weight was slightly lower in the high dose group, but the difference was not statistically significant. Developmental anomalies were present and included agenesis of the gall bladder and agenesis of the caudal lung lobe in fetuses from the high dose group. The incidence of fused sternbrae was also increased in the high dose group when compared to controls.

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The following table provides information on the external, visceral and skeletal observations in the fetuses.

Table IV: External Examinations

<u>Observations</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
#pups(litters) examined	190(21)	137(15)	143(19)	159(19)
<u>External Observations</u>	<u>Number(%) Affected</u>			
Forelimb Flexure	F: 0 L: 0	2(1.5) 1(6.7)	0 0	0 0
Umbilical Hernia	F: 0 L: 0	0 0	1(0.7) 1(5.3)	0 0
Omphalocele	F: 0 L: 0	0 0	0 0	2(1.3) 2(10.5)
Generalized Edema	F: 0 L: 0	0 0	0 0	1(0.6) 1(5.3)
Subdermal Hematoma	F: 0 L: 0	0 0	0 0	2(1.3) 2(10.5)

F = Fetuses affected, L = Litters affected

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Table IV (con't.): Visceral Examinations

Observations	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	190(21)	137(15)	143(19)	159(19)
<u>Visceral Observations</u>	<u>Number(%) Affected</u>			
Dilated Cerebral ventricles	F: 0 L: 0	1(0.7) 1(6.7)	1(0.7) 1(5.3)	0 0
Hydrocephaly	F: 0 L: 0	1(0.7) 1(6.7)	0 0	1(0.6) 1(5.3)
Patent ductus arteriosus	F: 1(0.5) L: 1(4.8)	0 0	0 0	0 0
Retroesophageal Rt. subcl. art.	F: 0 L: 0	0 0	0 0	2(1.3) 2(10.5)
Hypoplastic Lung Lobe	F: 1(0.5) L: 1(4.8)	0 0	0 0	0 0
Missing Caudal Lung Lobe	F: 2(1.1) L: 2(9.5)	2(1.5) 2(13.3)	0 0	5(3.1) 2(10.5)
Agenesis of the Gall bladder ^a	F: 2(1.1) L: 1(4.8)	1(0.7) 1(6.7)	1(0.7) 1(5.3)	13(8.2) 5(26.3)
Pale spleen	F: 0 L: 0	0 0	0 0	2(1.3) 2(10.5)
Dilated renal pelvis	F: 1(0.5) L: 1(4.8)	0 0	1(0.7) 1(5.3)	0 0
Retrocaval ureter	F: 4(2.1) L: 4(19.0)	0 0	1(0.7) 1(5.3)	0 0
Paraovarian Cyst ^b	F: 1(1.0) L: 1(4.8)	0 0	0 0	2(2.4) 2(10.5)
Multiple Cardiac Malformations	F: 0 L: 0	1(0.7) 1(6.7)	0 0	0 0

a = Agenesis of the gall bladder was present in 6/8 pups from one doe which experienced severe maternotoxicity after being exposed to 80 ppm of methyl bromide.

b = The percentages for the number of fetuses with paraovarian cysts were derived from the number of female fetuses examined.

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Table IV (con't.): Skeletal Examinations

<u>Observations</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
#pups(litters) examined	190(21)	137(15)	143(19)	159(19)
<u>Skeletal Observations:</u>				
<u>Skull</u>				
Delayed ossification	F: 2(1.5) L: 2(13.3)	7(3.7) 2(9.5)	2(1.4) 2(10.5)	12(7.5) 4(21.1)
Foramen	F: 3(2.2) L: 2(13.3)	1(0.5) 1(4.8)	3(2.1) 1(5.3)	8(5.0) 4(21.1)
<u>Hyoid</u>				
Delayed ossification	F: 37(19.9) L: 15(71.4)	42(30.9) 11(73.3)	24(17) 10(52.6)	41(26.3) 16(84.2)
Crooked	F: 3(1.6) L: 3(14.3)	2(1.5) 2(132.3)	2(1.4) 2(10.5)	0 0
<u>Dentoid Process</u>				
Delayed Ossification	F: 0 L: 0	3(1.6) 2(9.5)	0 0	2(1.3) 2(10.5)
<u>Pubis</u>				
Delayed Ossification	F: 6(4.4) L: 4(26.7)	3(1.6) 2(9.5)	1(0.7) 1(5.3)	13(8.2) 6(31.6)
<u>Spurs</u>				
Lumbar	F: 27(19.9) L: 11(73.3)	42(22) 14(66.7)	29(20.3) 13(68.4)	29(18.2) 14(73.7)
<u>Sternebrae</u>				
Delayed ossification	F: 67(49.3) L: 15(100)	60(31.4) 15(71.4)	50(35) 15(78.9)	49(30.8) 13(68.4)
Fused	F: 0 L: 0	0 0	3(2.1) 2(10.5)	20(12.6) 10(52.6)
<u>TOTALS</u>				
fetuses with malformations(%)	4(2.1)	6(4.4)	2(1.4)	23(14.5)
litters with malformations(%)	3(14.3)	6(40)	2(10.5)	12(63.2)

D. Discussion/Conclusions

Maternal Toxicity:

As stated earlier in the report, maternal toxicity was observed at exposure levels of 80 ppm and was characterized by clinical signs which included lethargy, right side head tilt, recumbency and ataxia. Decreased fecal output was also reported. One dam in the 80 ppm dose group delivered one day early and it was determined that this early delivery may have been related to the toxicity that this animal was experiencing.

Developmental Toxicity:

i. Deaths/Resorptions: No fetal deaths were reported in any of the groups. The number of resorptions were not statistically significant when treated animals were compared to controls.

ii. Altered Growth: The mean fetal weights were slightly lower in the high exposure group when compared to controls; however, the reported difference was not statistically significant.

iii. Developmental Anomalies: The most significant developmental anomalies reported were agenesis (absence) of the gall bladder in 8.2% of the fetuses in the high dose group agenesis of the caudal lung lobe in 3.1% of the fetuses in this same dose group. These figures in the 80 ppm group are compared to 1.1% for the concurrent control group and 0.9% for historical controls for agenesis of the gall bladder and 1.1% for concurrent controls and 0.05% for historical controls for missing caudal lung lobes.

When litter data are considered, the incidence of agenesis of the gall bladder was 26.3% in the high dose group and 4.8% in the control. The missing caudal lung lobe was not as significant when litters are compared. This anomaly occurred in 10.5% of the high dose litters and 9.5% of the control litters.

iv. Malformations: The number of fused sternbrae were increased in the high dose group (12.6%) when compared to controls (0). There was also an increase in the occurrence of delayed ossification of the pubis when compared to the controls. (8.2% at 80 ppm v 4.4% at 0 ppm). The increase in the incidence of fused sternbrae is probably related to the compound; however the delayed ossification of the pubis is probably incidental.

When all malformations are considered, the number of fetuses and the number of litters affected are higher for the group of animals exposed to 80 ppm methyl bromide (14.5% for 80 ppm; 2.1% for controls).

E. Study Deficiencies: No deficiencies were reported that would alter the validity of the study. The sponsor should address the observed discrepancies in the body weights reported for the high dose animals in the raw data and in the study summary.

F. Core Classification: Core Minimum

Maternal NOEL = 40 ppm
Maternal LOEL = 80 ppm
Developmental Toxicity NOEL = 40 ppm
Developmental Toxicity LOEL = 80 ppm

G. Additional Information:

In order to determine whether the observed finding of agenesis of the gall bladder was related to treatment or was directly related to the genetic make-up of one particular buck, an additional study was run in which does were mated with the suspect buck and left unexposed to methyl bromide. An additional group of does was inseminated with sperm from other bucks and these animals were exposed to methyl bromide at the 80 ppm level.

The results from this portion of the study revealed that four fetuses from the group of does exposed to 80 ppm methyl bromide had no gall bladders. Gall bladders were present in all fetuses from the group of does that were inseminated from the same buck and not exposed to methyl bromide. Skeletal specimens were not examined in this part of the study.

This study indicates that agenesis of the gall bladder is related to the test compound. This portion of the study also indicates that there is also a fetal weight decrement at 80 ppm that is also related to the test compound.